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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re U.S. Patent Application No. 10/628,464

Filed: July 29, 2003

Art Unit: 1646 Confirmation No. 4793

Title: IDENTIFICATION OF A NOVEL BITTER TASTE RECEPTOR, T2R76

**AFFIDAVIT BY MARK J. ZOLLER , Ph. D.**

I, Mark Zoller, declare and state as follows:

(1) That I am an inventor of the above-identified application..

(2) That I am currently employed. as the Chief Scientific Officer and Senior Vice President at Senomyx Inc., the Assignee of this patent application..

(3) That I reviewed the Office Action dated March 30, 2005 in the above-identified patent application relating to the hT2R76 a novel member of the T2R taste receptor family.

(4) That based thereon I understand that the Examiner has initially concluded that the as-filed specification contains insufficient evidence to reasonably that hT2R76 is a bitter taste receptor. I respectfully disagree.

(5) That experiments have been conducted at Senomyx Inc. under my supervision which have confirmed that hT2R76 encodes a bitter taste receptor that specifically binds to bitter ligands including PROP a bitter ligand specifically identified in our as-filed patent application

(6) (See example 6 of our patent application which lists 6 bitter ligands as being putative bitter ligands for hT2R76 including PROP. )

(7) That more specifically experiments were conducted using cell-based assays that detect changes in intracellular calcium concentration. In brief, using essentially the same fluorescent detection methods described in our patent application, human embryonic cells (HEK-293 cells) are initially seeded into 48-cell culture plates. 24 hours late, these cells are transiently transfected with a plasmid containing the hT2R76 nucleic acid sequence disclosed in our patent application along with a plasmid encoding a G protein (G16gust44). Another 24 hours later these cells are incubated with a fluorescent dye specific for calcium (Fluo-4; Molecular Probes) that provides a fast, simple and reliable fluorescent-based method for detecting changes in calcium concentration within the cell. [If a ligand specifically activates hT2R76, this elicits a signaling cascade, which leads to the activation of PLC and a subsequent increase in intracellular concentration. This increase in intracellular calcium concentration affects the fluorescent properties of the calcium specific dye within the cells. These changes may be monitored using the fluorescent microscopy imaging methods disclosed in our patent application using specifically designed software. (Imaging Workbench, Axon). ]

(8) That using these methods we screened HEK-293 cells expressing the above-identified hT2R76 sequence with a number of bitter ligands (including PROP, Brucine (a bitter alkaloid found in Strychnos seeds), L-tryptophan, salicin, and N-phenylthiourea.)

(9) That using these methods we observed that the PROP and Brucine bitter ligands both specifically activated hT2R76 expressed in HEK-293 cells, resulting in detectable changes in intracellular calcium levels (increase in fluorescence). By contrast, the other (control) bitter

ligands, including L-tryptophan, salicin, and phenylthiourea had no effect on intracellular calcium levels as evidenced by no detectable changes in fluorescence. (The results of these calcium imaging experiments are contained in Figure 2 attached to this affidavit.)

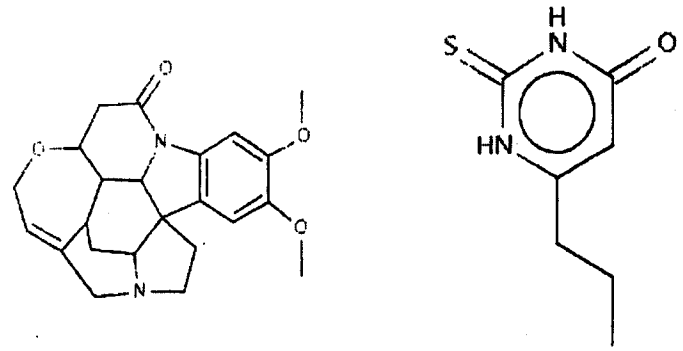
(10) That in my opinion the results of these experiments confirm what we reasonably anticipated on filing this patent application, i.e., that hT2R76 (SEQ ID NO:1) encodes a human bitter taste receptor which when screened against bitter ligands using the methods described herein and in our patent application would be shown to specifically respond to known and available bitter ligands such as PROP and Brucine. [Brucine is a well known bitter toxic alkaloid expressed in Strychnos seeds with a bitter taste threshold of 0.01 mM. Similarly, PROP is a well known bitter compound that elicits a bitter taste threshold of 0.01mM for PROP tasters].

(11) All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like may jeopardize the validity of the application or any patent issuing thereon.

9/28/05
Date

Mark J. Zoller

**Figure 1 Structures of Brucine and PROP**




Brucine PROP

**Figure 2 hT2R76 specifically responds to Brucine and PROP**